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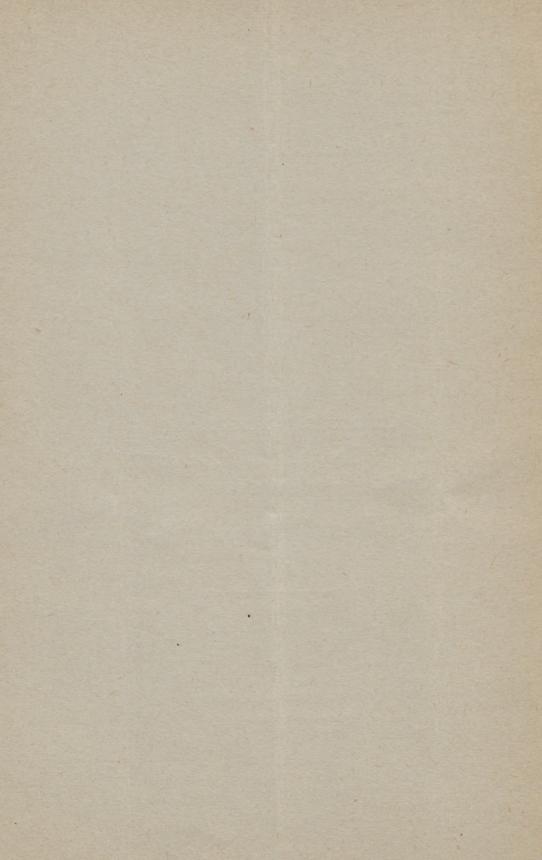
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presented by the author



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SOME OBSERVATIONS ON THE GROWTH OF DIATOMS IN SURFACE WATERS.

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For more than a century the study of the diatoms has been a fascinating pastime. Much has been written on the beauty of their form and markings, their animal or vegetable nature, their classification, and their peculiar spontaneous movements. Their study is now becoming one of practical importance, because it has been found that these little plants are often present in large numbers in the ponds and reservoirs of our public water supplies, and that they give rise to unpleasant tastes and odors in the water.

On account of the important effects produced by these and other micro-organisms, considerable attention is now being given to the microscopical examination of water. The most extensive series of such examinations thus far published are those of the Massachusetts State Board of Health and those made at the biological laboratory of the Boston Water Works. The former are valuable because of their wide range, covering as they do all the public water supplies of the State. The value of the latter lies in the fact that the examinations have been made at very frequent intervals, and that in every way the work has been carried into great detail. The temperature readings which accompany each sample are also of great value. It is the purpose of this article to review some of the data obtained from these two sources in regard to the Diatomaceæ, particularly with reference to their seasonal distribution.

² See Annual Reports of the Boston Water Board for the years 1890 to 1893, inclusive.



¹ See Annual Reports of the Massachusetts State Board of Health for the years 1890 to 1892, inclusive. Also special Report of the State Board of Health on Examinations of Water Supplies, 1890.

It will be but a slight digression for me to refer briefly to the biological work of the Boston Water Works. The Biological Laboratory was established at the Chestnut Hill Reservoir in the fall of 1880 by Mr. Desmond FitzGerald, Resident Engineer and Superintendent of the Western Division; and since January, 1890, the work has been carried on by the writer under his direction. So far as I am aware. this was the first laboratory established by any city for the purpose of making systematic biological examinations of its water supply. Ever since the work was inaugurated weekly examinations have been made of samples of water collected from all parts of the supply, i.e., from the brooks, ponds, storage reservoirs, and service taps. Thus we are enabled to give a history of the water from the time it falls from the clouds until it reaches the mouths of the consumers. Since the first of January, 1890, more than 12,000 microscopical and more than 5,000 bacteriological examinations have been made at the laboratory. Besides the regular examinations numerous special investigations are constantly being made. The laboratory is provided with an excellent dark room, and photomicrographs of most of the important microorganisms have been obtained.

The diatoms, or technically the Diatomaceae, are minute plants forming a group of microscopic Alga remarkable for their siliceous epiderm and for their variety of form and markings. They are unicellular, though in some genera the cells are united into filaments. The cell contents consist of a membrane, cell sap, nucleus, chromatophore plates, and sometimes oil globules and starch grains. Living diatoms are surrounded by a gelatinous envelope, which, on account of its transparency, can be seen only by adding coloring matter to the surrounding fluid. Of the cell contents biologists are at the present time most interested in the oil globules, because it is being proved that the oils present in the micro-organisms are the direct cause of many of the bad tastes and odors of certain drinking waters.

Of the one hundred and more genera into which the diatoms have been classified there are not more than twenty that are commonly found in our water supplies, and only six have, thus far, been found to be of practical importance, namely, Asterionella, Tabellaria, Melosira, Synedra, Stephanodiscus, and Diatoma. Some of the other genera occasionally met with are Cyclotella, Cymbella, Epithemia, Fragilaria, Gomphonema, Meridion, Navicula, Nitzschia, Pleurosigma, Schizonema, Stauroneis, and Surirella.

The six most important genera are not always observed in the same reservoir. Generally there are certain diatoms peculiar to certain ponds. Lake Cochituate, for instance, often contains large growths of Asterionella, Tabellaria, and Melosira, and smaller growths of Synedra and Stephanodiscus. Basin No. 3 contains Asterionella, Tabellaria, and Synedra, but no Stephanodiscus nor Melosira. In Basin No. 2 only Synedra and Cyclotella are found. Fresh Pond, Cambridge, is famous for its Stephanodiscus, and Diatoma is often very abundant in the Lynn waters. Furthermore, there are ponds where diatoms are never found, except in very small numbers at rare intervals, while in neighboring ponds they may be present in such large numbers that a bottle of the water, when held towards the light, has a silvery, glistening appearance.

Just why diatoms grow in some ponds and not in others it is at present impossible to say. A suggestion as to the reason will be offered later on in this paper, and as the local distribution is found to be closely connected with the seasonal distribution these two subjects will be treated together.

In an article by G. N. Calkins,¹ on "The Seasonal Distribution of Microscopical Organisms in Surface Waters," it has been shown that there are two seasons of the year, namely, the spring and the late fall, when the diatoms increase prodigiously. The spring maximum occurs in April, and the fall maximum in December. During the summer and the greater part of the winter diatoms are sometimes found, but only in small numbers.

My own observations on the reservoirs of the Boston Water Works confirm these results. I have noticed that, while it is true that diatoms appear with considerable regularity each spring and fall, the genera which appear at any given season are not always the same. If we consider, for example, the spring growths in Lake Cochituate, we find that in 1890 the Asterionella first appeared, and that this growth was soon followed by one of Tabellaria. In 1891 the growth was chiefly Asterionella, Melosira appearing about the same time but not developing to any great extent. In 1892 Melosira was the predominant diatom; in 1893, Melosira and Asterionella; and in 1894, Tabellaria, Asterionella, and Melosira. It would be a matter of great

¹ G. N. Calkins: The Seasonal Distribution of Microscopical Organisms in Surface Waters. Twenty-fourth Annual Report Massachusetts State Board of Health, 1892.

interest to be able to account for these occurrences; we might then be able to control the conditions which favor the growth of one genus and retard the growth of another. We should like to keep down the *Asterionella*, the diatom which is most active in producing tastes and odors in the water. In case we could not get rid of the diatoms entirely, we would willingly accept a growth of *Synedra* if we could keep out the *Asterionella* and *Tabellaria*. So far as I know, *Synedra* has never been the cause of any trouble.

A comparison of the microscopical examinations of the different reservoirs of the Boston Water Works shows that Basins No. 2 and

TABLE NO. I.

Amorphous Matter.

Average of weekly examinations of samples from the surface, mid-depth, and bottom for a period of four years.

Locality.	Number of Standard Units 1 per cc.
Lake Cochituate	432
Basin 3	407
Basin 2	322
Basin 4	228

No. 4 never have extensive diatom growths, but that in Basin No. 3 and Lake Cochituate these plants develop regularly in the spring and fall. It is possible that the reason for this difference lies in the different conditions of the bottoms of these reservoirs. When Basin No. 4 was constructed all the peat, turf, etc., was carefully removed from the bottom. This was done over a large part of Basin No. 2. In Basin No. 3 the stripping was not as thoroughly done, and the bottom was originally more swampy. Lake Cochituate is a deep, natural pond with a muddy bottom. On account of this soft bottom with its organic matter the "amorphous matter" has always been higher in Lake Cochituate than in Basins No. 2 and No. 4. This is shown by Table No. I.

¹ One Standard Unit represents a surface area of four hundred square microns.

The fact that the diatom growths are in some way connected with the muddy bottoms of ponds is also suggested by their seasonal distribution. It has been observed that the spring growths occur at about the same time in Basin No. 3 and in Lake Cochituate, but that the fall growths always take place later in Lake Cochituate than in Basin No. 3. The reason for this is to be found in the phenomenon of "stagnation."

It is well known that in ponds which are more than 25 or 30 feet deep the temperature of the water at the bottom remains quite constant during the summer, while the temperature of the surface water rises and falls with the temperature of the air. The consequence is that the lower strata of water remains stagnant during the summer; that is to say, there are no vertical currents in the water below the depth where the wind ceases to keep the water in motion. In the fall the surface water cools until it reaches the same temperature as the water at the bottom. Then when the density of the water is the same at all depths there is a stirring up; the lower layers are brought to the surface, and the light, flocculent, amorphous matter, always abundant at the bottom when the soil is muddy, is distributed through the water. During the winter, when the surface of the water is frozen, there is another period of stagnation, due to the fact that the temperature of the water at the bottom tends to remain at the point of maximum density (39.2°F.), while the surface temperature sinks nearly to the freezing point. The winter stagnation takes place in both deep and shallow ponds.

The stagnation of the lower layers is also indicated by the color of the water. In Lake Cochituate the water at the bottom acquires a dark reddish brown color during the stagnation periods, most marked, of course, during the summer, when the period is long. At the time of the "turning over" this dark water is distributed through the vertical, the result being to increase the color at the surface. These facts are shown by diagram on Plate I.

It will be noticed that there are two periods of the year, each about six weeks long, when the water is in circulation from top to bottom. It is during these periods that the diatoms develop. Microscopical examinations have shown that both in Basin No. 3 and in Lake Cochituate the diatom growths occur soon after stagnation ends. The Asterionella, for instance, generally appears about one week after the turning over. It then increases, reaching its maximum growth in from twenty-five to fifty days.

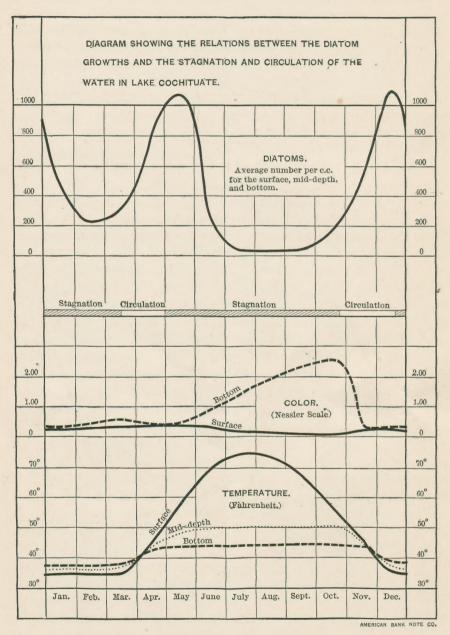


PLATE I.

The bottom temperature of Lake Cochituate is considerably lower than the bottom temperature of Basin No. 3 during the summer months, on account of its greater depth; hence its turning over occurs later in the year. This explains why the fall growth of diatoms occurs later in Lake Cochituate. The drawing down of Basin No. 3

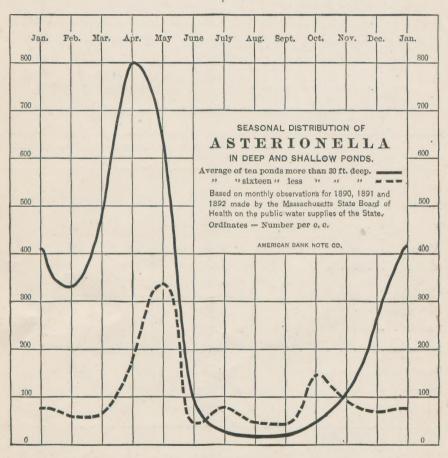


PLATE II.

each season for the supply of the city also affects the time of the turning over of that reservoir.

The examinations of the State Board of Health furnish corroborative evidence that the seasonal distribution of diatoms is controlled by the circulation and stagnation of the water. This may be seen by comparing the diatom growths in deep and shallow ponds. On the assumption that diatoms grow best immediately after the turning over, we should expect to find in the deep ponds two periods of diatom growth — one in the spring, following the winter stagnation; and one in the fall, after the summer stagnation. In the case of shallow ponds, however, we should expect to find a spring growth following the winter stagnation, and for the rest of the year a uniform or irregular distribution. This is found to be the case. Of twelve ponds and reservoirs more than 30 feet deep, eleven show a well-defined spring and

TABLE NO. II.

AVERAGE NUMBER OF ASTERIONELLA IN A CUBIC CENTIMETER OF WATER.

Based on Monthly Observations for Three Years.

	Month.	Average of Eleven Ponds and Reservoirs over 30 feet deep.	Average of Sixteen Ponds and Reservoirs less than 30 feet deep.
January .		 410	77
February		 331	58
March .		 475	61
April		 799	190
May		 617	332
June		 89	48
July		 29	75
August .		 27	46
September		 26	42
October .		 47	138
November		 103	93
December		 275	68

fall growth, while in one instance the growth was uniformly distributed; and of seventeen ponds and reservoirs less than 30 feet deep, eleven have diatom growths appearing at irregular intervals but having a slight spring maximum, while four have both a spring and fall growth.

Table No. II shows a comparison between the deep and shallow ponds with regard to the number of *Asterionella* found each month. The figures were obtained by averaging monthly observations extend-

ing over a period of three years. The first column is made up of averages of eleven ponds over 30 feet deep, and the second column of averages of sixteen ponds less than 30 feet deep. These results are shown by diagram on Plate II. The depth of 30 feet was selected as the dividing line between the deep and shallow ponds because stagnation does not ordinarily occur in ponds having less depth than that. It is probable, however, that the four shallow ponds referred to in the preceding paragraph as having spring and fall growths of diatoms have also periods of winter and summer stagnation.

An examination of Plate II shows that the diatom growths are most extensive in deep ponds. In those ponds, also, the spring and fall maxima are the most marked, and there is almost an entire absence of diatoms during the summer months. The spring growth is observed in the shallow ponds, and there is a slight indication of a fall growth. During the summer the curve for the shallow ponds is higher than for the deep ponds, and is somewhat irregular. It will also be noticed that in the deep ponds the spring growth occurs earlier and the fall growth later than in the shallow ponds. This is what might be expected from what we know of the phenomenon of stagnation.

In order to find the reason for the rapid development of diatoms after the turning over of the water, let us look at some of the chemical analyses. In Table No. III will be found a summary of the State Board of Health analyses for Lake Cochituate and Basins 2, 3, and 4 for a period of about five years.

Bearing in mind that Lake Cochituate and Basin No. 3 support immense growths of diatoms and that Basins No. 2 and No. 4 do not, it will be seen that the mineral constituents as indicated by the chlorine, fixed residue, hardness, and nitrates are higher in those cases where diatoms abound. The free ammonia and nitrites are also somewhat higher. On the other hand, the color, loss on ignition, and albuminoid ammonia do not seem to bear any direct relation to the diatom growths.

Reasoning by what is known of the higher plants, we may assume that the most important factor is that of the nitrates, and we should expect, therefore, that the nitrates would be high during or just preceding a growth of diatoms.

That this is the case is shown by Table No. IV, where the chemical analyses are tabulated by periods corresponding to the rise, fall, and absence of diatoms. Each of these periods is subdivided. The first

subdivision includes the time when the surface of the water was frozen; and the second, the period of open water. This subdivision is important because it has been found that no extensive growths of diatoms occur when the surface of the water is covered with ice.

During the period of diatom development the nitrates were .0240 in Lake Cochituate, and .0200 in Basin No. 3. While the diatoms were disappearing, yet above 200 per cc., and when there were fewer

TABLE NO. III. CHEMICAL ANALYSES. Means of Monthly Analyses for Five Years. (Parts per 100,000.)

	Lake Cochituate.	Basin 3.	Basin 2.	Basin 4.
Color	.20	.75	.89	.67
Residue, total	4.66	5.07	4.31	3.51
Residue, loss on ignition	1.35	1.90	1.82	1.53
Residue, fixed	3.32	3.18	2.48	1.99
Chlorine	.46	.39	.28	.22
Albuminoid Ammonia, suspended	.0034	.0045	.0038	.0034
Albuminoid Ammonia, dissolved	.0144	.0215	.0201	.0176
Free Ammonia	.0018	.0026	.0008	.0011
Nitrites	.0002	.0002	.0001	.0001
Nitrates	.0113	.0171	.0085	.0065
Hardness	2.07	1.80	1.05	1.19

than 200 per cc., the nitrates were high during the time when the surface was frozen; but they were much lower during the period of open water. This seems to indicate that diatoms require both nitrates and a free circulation of air. If either is lacking they will not develop. This fact throws some light on their seasonal distribution. In the winter air is lacking; in the summer nitrates are low; but in the spring and fall nitrates and air are both present. That diatoms need air has already been shown by laboratory experiments, but whether it is the oxygen or the carbonic acid gas of the air has not been determined.

It is probable, also, that the effect of light has some bearing on

TABLE NO. IV.
CHEMICAL ANALYSES. (Parts per 100,000).

	Condition of	Fixed Solids.	olids.	Albuminoid Ammonia (Filtered).	Ammonia ed).	Free Ammonia.	monia.	Nitrites.	es.	Nitrates.	es.
Diatoms.	Surface.	Lake Cochituate.	Basin 3.	Lake Cochituate.	Basin 3.	Lake Cochituate.	Basin 3.	Lake Cochituate.	Basin 3.	Lake Cochituate.	Basin 3.
Increasing	Frozen.	•			:		:	* * * * * * * * * * * * * * * * * * *	•	•	:
Increasing	Not frozen.	3.44	2.92	.0143	.0217	.0027	.0016	.0002	.0001	.0240	.0200
Above 200 per cc., but decreasing	Frozen.	3.62	:	.0141	•	.0026	• • • • • • • • • • • • • • • • • • • •	.0002	# # #	.0280	:
Above 200 per cc., but decreasing	Not frozen.	3.00	3.26	.0137	.0256	.0011	.0010	.0003	.0002	.0110	.0103
Below 200 per cc	Frozen.	3.15	3.25	.0129	.0142	.0007	.0043	.0002	.0002	.0215	.0296
Below 200 per cc	Not frozen.	3.27	3.22	.0147	.0210	.0007	.0020	.0003	.0002	.0104	9600.

their growth. It has been proved that diatoms will not develop in the dark, and it is said that bright sunlight will kill them.

Table No. IV also shows that the fixed solids, dissolved albuminoid ammonia, and the free ammonia bear little relation to the diatom growths. If, now, we compare Table No. IV with Table No. V, which gives the amount of nitrates present in Basins No. 2 and No. 4 during the periods corresponding to the increase, decrease, and absence of diatoms in Basin No. 3, we shall see, perhaps, why these basins do not support large diatom growths. When the diatoms were increasing in

TABLE NO. V.
CHEMICAL ANALYSES. (Parts per 100,000.)

Decree of Brown	Condition of Surface of	Niti	rates.
Diatoms in Basin 3.	Basins 2 and 3.	Basin 2.	Basin 4.
Increasing	Frozen.		
Increasing	Not frozen.	.0110	.0070
Above 200 per cc., but decreasing	Frozen.		
Above 200 per cc., but decreasing	Not frozen.	.0080	.0018
Below 200 per cc	Frozen.	.0160	.0108
Below 200 per cc	Not frozen.	.0051	.0058

Basin No. 3, the nitrates were only .0110 in Basin No. 2 and .0070 in Basin No. 4, as compared with .0200 in Basin No. 3 and .0240 in Lake Cochituate, and during two of the other periods the nitrates were still lower. During one period they rose to .0160 in Basin No. 2 and .0108 in Basin No. 4; but at that time the surface was frozen and there was no circulation of air, and consequently no growth.

As a confirmation of the theory that diatoms require nitrate food we have the following analyses from the State Board of Health. Eighteen ponds and reservoirs in which Asterionella growths reached 1,000 per cc. gave .0188 as the yearly average of the nitrates present; six ponds and reservoirs where the growth was between 500 and 1,000 per cc. gave an average of .0091; and for sixteen ponds and reservoirs where the Asterionella were less than 500 per cc. the average of the nitrates was .0080. Of the eighteen ponds and reservoirs which make

up the average .0188, five were less than .0080; but these ponds had the nitrates considerably above .0100 during the periods when the diatoms were increasing.

It must not be supposed, however, that the diatoms live entirely on nitrates. Other food materials are, of course, necessary; and it is possible that the diatoms may have the power of assimilating nitrogen in other forms than the nitrates. Culture experiments are now being conducted at the laboratory of the Boston Water Works which may throw some light on this question.

We have now to consider the condition of the water at the bottom of the deep ponds. This is shown by Table No. VI, where analyses are given for the surface and bottom of Lake Cochituate, Basins No. 3 and No. 4, and Jamaica Pond during the stagnation periods. In Lake Cochituate, Basin No. 3, and Jamaica Pond there is seen to be a large amount of decaying organic matter at the bottom. This is shown by the high free ammonia. On account of the absence of oxygen at the bottom the decomposition could not be carried beyond that stage. Iron, manganese, and silicon were also highest at the bottom. In the case of Basin 4, which was stripped of its peat, loam, etc., before filling, there is little decomposable organic matter at the bottom.

At the turning over of the water the free ammonia is brought to the surface, where the bacteria, supplied with plenty of oxygen, complete the decomposition, changing the nitrogen to the nitrate form. At the same time, also, it is probable that the diatoms or their spores are brought up from the bottom and scattered through the water, where, finding an abundance of nitrates and air, they develop rapidly. If, however, there is no free ammonia at the bottom, as is ordinarily the case in properly prepared reservoirs, no nitrates will be found at the time of the turning over, and consequently no diatom growths will appear.

We thus have strong evidence in favor of the removal of the top soil when preparing a reservoir for the storage of water. This position has already been taken by some of the most eminent of our engineers. Its practice ought to be universal.

The question of the rate of increase of micro-organisms is an important one. It is especially interesting in the case of diatoms, because it has been the subject of numerous controversies. It has generally been assumed that the increase takes place in accordance with the law of geometrical progression, *i.e.*, starting with a single

TABLE NO. VI.

CHEMICAL ANALYSES. (Parts per 100,000).

			on I	Residue Evaporation.	e ation.			Z	NITROGEN.	.4				Dissolved Oxygen.			
Locality.	Date.			ition.			Albuminoid Ammonia.	ninoid onia.	nia.			sumed.		Sat.			
		Color.	Total.	Loss on Ign	Fixed.	Chlorine.	Unfiltered.	Filtered.	Free Ammo	As Nitrites.	As Nitrates.	Oxygen Con	Hardness.	Per cent. S	Iron.	Manganese.	Silica.
Lake Cochituate, Surface	Sept. 28, 1891.	.12	4 70	1.15	3.55	.44	,0134	9110	.0002	.0002	.0020	.34	1.82	100	.II	.04	.31
Lake Cochituate, Bottom (65 feet) .	Sept. 28, 1891.	3.40	8,05	2.25	5.80	.42	.0244	.0186	.0736	.0005	.0020	1.07	1.98	0	.96	.21	.86
Lake Cochituate, Gate House	Nov. 2, 1891.	*25	4.60	1.10	3.50	.46	.0144	.00100	.0044	1000.	.0200	:	1.9	:	:	:	:
Basin No. 3, Surface	Aug. 19, 1891.	.35	6.25	2.40	3.85	.45	.0224	.0214	.0008	.0002	.0020	.65	1.55	85.88	.17	.03	:
Pasin No. 3, Bottom (25 feet)	Aug. 19, 1891.	3.00	7.20	2.45	4.75	.44	.0310	.0250	.0496	.0004	.0020	1.20	1.96	0	.70	.16	:
Basin No. 4, Surface	Aug. 19, 1891.	.42	3.65	1.80	1.85	.25	.0134	.0122	.0002	.0002	.0030	.73	1.14	84.50	.06	.03	:
Basin No. 4, Bottom (35 feet)	Aug. 19, 1891.	-55	3.20	1.45	1.75	.20	.0118	oiio.	.00008	.0002	.0040	.68	1.22	15.10	.22	.06	:
Basin No. 4, Surface	Oct. 1, 1891.	•35	3.25	1.85	1.40	.23	.0178	.0146	.00000	.0000	.0070	:	.09	:	:	:	:
Jamaica Pond, Surface	Aug. 14, 1890.	:	:	:	:	:	.0362	.0220	.0000	.0000	.0030	:	:	:	:	:	:
Jamaica Pond, Bottom (50 feet)	Aug. 14, 1890.	:	:	:	:	:	.0460	.0350	.4720	.0000	.0150	:	:	:	:	:	:
Jamaica Pond, Surface	Nov. 27, 1889.	:	:	:	:	:	.0246	osio,	.0640	,0009	.0120	:	:	:	:	:	:
Jamaica Pond, Bottom (50 feet)	Nov. 27, 1889.	:	:	:	:	:	.0236	.0174	.0712	.0009	.0150	:	:	:	:	:	:
													-				-

cell, this cell after a certain time divides into two, each of which after another interval of time divides into two more, and so on, so that after n intervals of time, the number of cells would be ar^n , where a = 1, and r = 2.

There are some writers, following Otto Müller, who have claimed that the increase takes place more slowly than this, because, they say, when a cell divides the smaller of the resulting cells does not have the same power of reproduction as the larger on account of lacking the necessary thickness of connective band. My own observations seem to show that the diatoms do increase substantially in accordance with the laws of geometrical progression, and that the ratio varies between 1.3 and 2.0 per week, the average ratio for ten growths of Asterionella covering a period of thirty-five weeks being 1.58. The corresponding ratio for Tabellaria was 1.56. We may say, in a general way, that during a vigorous growth the diatoms increase at the rate of about 50 per cent. each week.

On Plate III will be found a diagram showing the rate of growth of Asterionella in Lake Cochituate. The full lines represent the diatom growths. The broken line represents the equation $l = ar^n$, where a = 1, r = 1.58, and n = the number of weeks. The number of weeks are plotted as abscissæ, and the number of Asterionella per cc., as ordinates.

A growth of *Synedra* in Basin No. 2, lasting ten weeks, had a slower rate, the average ratio being 1.3 per week. In this growth the *Synedra* multiplied more rapidly at first than they did later on. The ratio for the first half was 1.4, and for the second half, 1.2.

But to say that the diatoms increase slowly and regularly does not tell the whole story. Oftentimes they develop with extraordinary rapidity, sometimes jumping from 132 to 1,575 per cc. in a single week, as Asterionella did in Walden Pond, Lynn, Massachusetts, in October, 1893. The reason for these sudden and enormous developments is not known. We have tried to associate them with some sudden increase in the amount of food material. But though we have been thus far unsuccessful, it is quite probable that that is the cause. It is possible, as some have suggested, that it is in some way connected with the formation of sporangial frustules, the result of conjugation.

¹ Dr. H. Van Heurck. Synopsis des Diatomées en Belgique, p. 12.

This phenomenon is an interesting one, but comparatively little is known about it. The theory is this. The diatoms grow smaller as they subdivide. Finally, after reaching a certain size, or after a certain weakening of the power of subdivision, a conjugation takes place

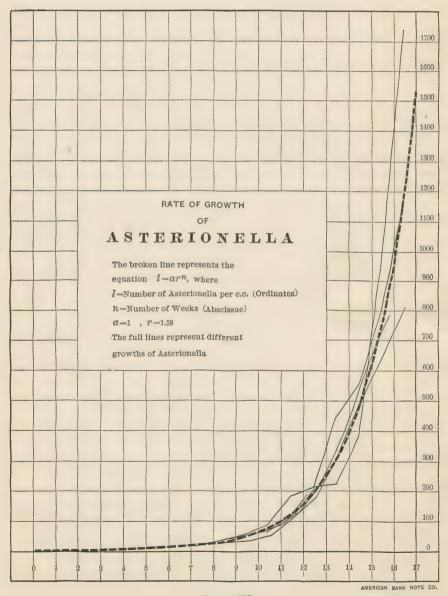


PLATE III.

between two of the cells, the result being a sporangial frustule, from which a larger cell is produced. This divides and subdivides, the new cells growing smaller and smaller, until a new conjugation is necessary. Among certain genera artificially cultivated this process has been observed. I have found the sporangial frustules of *Melosira* in a jar of water which had been standing for a few weeks in the laboratory, and I have seen *Asterionella* in what appeared to be a conjugating form; but the facts do not warrant any positive statements. The decrease in the size, however, has been repeatedly observed; it is especially marked in *Stephanodiscus* and *Cyclotella*.

Thus far we have considered only the increase of the diatoms. They probably cease to develop when their food supply is cut off. This has already been shown by Table No. IV. The periods of decreasing growth are marked by a deficiency of air or nitrates. It is to be regretted that more determinations of nitrates have not been made in connection with the development of diatoms. With frequent observations it might be possible to predict the extent of growth and the time when the growth would culminate. Such information might be of practical value.

The decrease of diatoms is ordinarily quite abrupt, but they sometimes linger over a long period. Generally a rapid growth is followed by a rapid fall. During the period of decline the cells are often broken up; the brown coloring matter is less abundant, and is frequently massed together in spots instead of being spread out in plates over the cell; occasionally it has a slight greenish color. Empty cells are always abundant during the declining period. During an increasing growth the diatoms are generally most abundant at the surface of a pond; during the period of decline they are usually more abundant towards the bottom.

As to the effect of temperature on diatoms, our observations indicate that the variations of temperature usually met with in the ponds of this climate have comparatively little influence on their growth, certainly not enough to account for their seasonal distribution. Diatoms grow well both in summer and winter, provided food is plenty. Vigorous growths have been observed at temperatures ranging from 35° to 75° F. The mean temperature at which the maximum Asterionella growths have been observed in Lake Cochituate is 50° F., the temperatures of the different growths, however, varying from 35° to 67°. The temperature of the water affects the diatoms indirectly by producing stagnation, as has already been pointed out.

Résumé.

In this paper I have endeavored to show:

- I. That the growth of diatoms in ponds is directly connected with the phenomenon of stagnation; that their development does not occur when the lower strata of water are quiescent, on account of greater density, but rather during those periods of the year when the water is in circulation from top to bottom.
 - 2. That diatoms flourish best in ponds having muddy bottoms.
- 3. That in deep ponds there are two well-defined periods of growth—one in the spring and one in the fall; that in shallow ponds there is usually a spring growth but no regular fall growth, and that other growths may occur at irregular intervals as the wind happens to stir up the water.
- 4. That the two most important conditions for the growth of diatoms are a sufficient supply of nitrates and a free circulation of air, and that both these conditions are found at those periods of the year when the water is in circulation.
- 5. That while temperature has possibly a slight influence on the growth of diatoms, it is of so little importance that it does not affect their seasonal distribution.
- 6. That the increase of diatoms takes place substantially in accordance with the law of geometrical progression, and that the cessation of their growth is caused by the diminution of their food supply.

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